resistant and susceptible populations when tests are incubated at 37°C (data not shown). Although presently not recommended for CoNS an evaluation of the method for testing these organisms is currently in progress.

References


Disc methods for detecting AmpC β-lactamase-producing clinical isolates of Escherichia coli and Klebsiella pneumoniae

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Sir,

The increasing prevalence of AmpC β-lactamase-mediated resistance among Escherichia coli and Klebsiella pneumoniae is of clinical concern.1 Both of these organisms can acquire AmpC β-lactamases on plasmids and additionally, E. coli can hyper-produce its chromosomal AmpC β-lactamase, which is normally only produced at very low levels.2 The recognition of AmpC β-lactamase producers can be difficult, although resistance to cefoxitin can help in identifying them. Unfortunately, cefoxitin resistance is not only due to AmpC β-lactamase production, but may also be due to decreased permeability. Several methods, based on the ability of cell-free extracts of organisms to hydrolyse cefoxitin, have been proposed as confirmatory tests for the production of AmpC β-lactamases.2,3 Most are too time-consuming for use in routine diagnostic laboratories and may not detect all AmpC β-lactamases.4 There is a need, therefore, for alternative methods that can be integrated into diagnostic laboratories and ideally do not rely on cefoxitin as the indicator antibiotic. We report our preliminary findings with a combination disc susceptibility method that uses cefpodoxime as an indicator and the AmpC β-lactamase inhibitor benzo(b)thiophene-2-boronic acid (BZBTH2B).1,5

Sixty non-replicate AmpC β-lactamase-producing clinical isolates of E. coli (n = 57) and K. pneumoniae (n = 3), collected from City Hospital, Birmingham (August 2001–March 2004) were tested. All of the isolates hydrolysed cefoxitin, as determined by the method of Nasim et al.3 and their cefoxitin MICs, as determined by the BSAC standardized agar dilution method,6 were reduced ≥4-fold in the presence of a fixed 100 mg/L concentration of cefoxitin.7 Fourteen of the isolates were positive by PCR for plasmid-borne bla<sub>ampc</sub> using primers and conditions described by Pérez-Pérez and Hanson.8 Seventy cefoxitin-resistant, AmpC β-lactamase-negative isolates of E. coli (n = 50) and K. pneumoniae (n = 20) were used as negative controls. All isolates were identified by API 20E (bioMérieux, Basingstoke, UK). Six laboratory strains of E. coli producing known AmpC β-lactamases (BIL-1, ACC-1, ACT-1, MIR-1, FOX-4, CMY-2) were also tested.

Combination discs were produced in-house using commercially available susceptibility discs (Oxoid, Basingstoke, UK and Mast, Bootle, UK) to which the inhibitor BZBTH2B was added. Stock solutions of BZBTH2B (VWR International Ltd, Lutterworth, UK) were made in DMSO and further diluted in 0.1 M NaOH and water. BZBTH2B was added to discs containing cefpodoxime 10 μg or cefpodoxime 10 μg + clavulanic acid 1 μg to give 64 μg BZBTH2B per disc. Blank discs containing inhibitor alone were used to check for possible intrinsic antimicrobial activity. IsoSensitest agar plates were inoculated with the test organisms to give semi-confluent growth using the BSAC standardized disc susceptibility method. Cefpodoxime and cefpodoxime + clavulanic acid discs with and without BZBTH2B inhibitor, and discs with the inhibitor alone, were spaced over the agar surface. After overnight incubation in air at 35–37°C, the zones of inhibition were measured. For comparison, the method was repeated using cefoxitin 30 μg discs with and without BZBTH2B (64 μg) or cloxacillin (100 μg).

A ≥5 mm increase in the zone diameter around the combined disc compared with that for cefoxitin, cefpodoxime or cefpodoxime + clavulanic acid alone, was considered significant. Using this cut-off, none of the combined discs gave a positive result with the cefoxitin-resistant, non-AmpC β-lactamase-producing isolates. BZBTH2B alone gave no zones of inhibition with any of the organisms tested. The combined disc methods cefoxitin + cloxacillin, cefoxitin + BZBTH2B, cefpodoxime + BZBTH2B and cefpodoxime + clavulanic acid + BZBTH2B correctly identified 57 (86.4%), 59 (89.4%), 64 (97%) and 66 (100%) of the AmpC β-lactamase producers, respectively. Significantly, the cefoxitin-based disc methods failed to detect the ACC-1 β-lactamase-producing strain, which is cefoxitin susceptible. ACC-1 β-lactamase-producing clinical isolates of both E. coli and K. pneumoniae have been described.4 Cefpodoxime + BZBTH2B did not detect two isolates, which concomitantly produced an AmpC β-lactamase and an extended spectrum β-lactamase (ESBL). The activity of the ESBL masked the effects of BZBTH2B. Cefpodoxime + clavulanic acid + BZBTH2B was the only method to detect all the AmpC β-lactamase producers, as the addition of clavulanic acid inhibited the activity of the ESBLs. This test is simple enough to be easily integrated into routine diagnostic laboratories and has the potential to greatly simplify the detection of AmpC β-lactamases in E. coli and K. pneumoniae.
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References


Bacterial prostatitis due to Pseudomonas aeruginosa harbouring the blaVIM-2 metallo-β-lactamase gene from Saudi Arabia

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The emergence of acquired metallo-β-lactamases (MBLs) of the IMP and the VIM types among Gram-negative bacilli is increasing and becoming a life-threatening therapeutic problem. These enzymes are active against most β-lactams including carbapenems and extended-spectrum cephalosporins except the monobactam aztreonam. Moreover, most of the clinical isolates harbouring such enzymes are also often resistant to other antibiotics such as aminoglycosides and fluoroquinolones. Therefore, antibiotic therapy for treating infections arising from MBL-producing strains constitutes a real therapeutic problem. MBLs belonging to the VIM family have been reported in European, Asian and American countries among strains of Pseudomonas aeruginosa, but also among strains of members of the family Enterobacteriaceae. blaVIM genes are often carried by mobile gene cassettes inserted in class 1 integrons chromosomally or plasmid located.2,3,7

P. aeruginosa RZ01 strain was recovered from an HIV-positive 64-year-old man, coming from Saudi Arabia who had been hospitalized for an abdominal mass due to a Cryptococcus neoformans voluminous abscess treated with amphotericin B and fluconazole and surgery. Empirical antibiotic combination of ceftriaxone (2 g/day) and trimethoprim/sulfamethoxazole (80/400 mg/day) was also administered at the same time. One week after the surgical procedure, and 10 days of urinary tract catheter, the patient developed a prostatitis and his condition worsened. P. aeruginosa RZ01 was isolated from urine and was resistant to most anti-pseudomonal β-lactams, including ticarcillin, piperacillin, ceftazidime, cefepime and imipenem, but was moderately susceptible to aztreonam. The isolate was also resistant to most other available antibiotics such as aminoglycosides and fluoroquinolones, except to colimycin and fosfomycin. MICs of β-lactams, determined by Etest and interpreted as recommended (AB Biodisk), were as follows: ticarcillin, 1024 mg/L; piperacillin, >1024 mg/L; ceftazidime, 64 mg/L; imipenem, >32 mg/L; meropenem, 24 mg/L; and aztreonam, 8 mg/L. By an agar dilution technique, the MIC of imipenem was found to be 256 mg/L. A synergy was found between imipenem or ceftazidime and EDTA-containing discs indicating that P. aeruginosa RZ01 produced an MBL. Repeated attempts to transfer ceftazidime or imipenem resistance by conjugation with filter mating to P. aeruginosa or Escherichia coli recipients were unsuccessful. Analysis of the plasmid content of the strain P. aeruginosa RZ01 did not reveal the presence of plasmid DNA and electroporation experiments failed. We used two sets of primers previously reported enabling the detection of class B β-lactamases genes.8 PCR experiments followed by sequencing revealed the presence of the carbapenemase VIM-2. The precise genetic environment of blaVIM-2 was not precisely determined except that using primers VIM-2A and VIM-2B, we were able to amplify 2.7 kb suggesting that this class one integron possesses other antibiotic resistance gene cassettes. Furthermore, we also demonstrated by PCR that blaVIM-2 was the first gene located downstream of intI1 suggesting that the molecular organization of this integron differs from that previously described in P. aeruginosa harbouring blaVIM-2.2,4,7

In vitro studies of antibiotic associations revealed that the combination of aztreonam plus fosfomycin was synergic and was the most effective of all associations tested. Antibiotherapy combining fosfomycin (12 g/day) and aztreonam (6 g/day) was initiated and continued for 21 days, the patient recovered and all subsequent urine cultures were sterile. The use of high doses of aztreonam was previously suggested to be the most appropriate

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